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A survey of xerophilic *Aspergillus* from indoor environment, including descriptions of two new section *Aspergillus* species producing eurotium-like sexual states

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Abstract

Xerophilic fungi grow at low water activity or low equilibrium relative humidity and are an important part of the indoor fungal community, of which *Aspergillus* is one of the dominant genera. A survey of xerophilic fungi isolated from Canadian and Hawaiian house dust resulted in the isolation of 1039 strains; 296 strains belong to *Aspergillus* and represented 37 species. Reference sequences were generated for all species and deposited in GenBank. *Aspergillus* sect. *Aspergillus* (formerly called *Eurotium*) was one of the most predominant groups from house dust with nine species identified. Additional cultures deposited as *Eurotium* were received from the Canadian Collection of Fungal Cultures and were also re-identified during this study. Among all strains, two species were found to be new and are introduced here as *A. mallochii* and *A. megasporus*. Phylogenetic comparisons with other species of section *Aspergillus* were made using sequences of ITS, β-tubulin, calmodulin and RNA polymerase II second largest subunit. Morphological observations were made from cultures grown under standardized conditions. *Aspergillus mallochii* does not grow at 37 °C and produces roughened ascospores with incomplete equatorial furrows. *Aspergillus megasporus* produces large conidia (up to 12 µm diam) and roughened ascospores with equatorial furrows. Echinulin, quinolactacin A_1 & A_2, preechinulin and neoechinulin A & B were detected as major extrolites of *A. megasporus*, while neoechinulin A & B and isoechinulin A, B & C were the major extrolites from *A. mallochii*.

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Key words
BenA, *CaM*, indoor environments, mycotoxin, *RPB2*

Introduction

Species of *Aspergillus* section *Aspergillus*, the "*A. glaucus*" group of Thom and Raper (1941) and Raper and Fennell (1965), typically produce yellow cleistothecia (white in *A. leucocarpus*) with lenticular ascospores and the section includes species that were traditionally classified in the genus *Eurotium*. Species of section *Aspergillus* have a broad distribution in nature, but their xerophilic physiology makes them significant for the built environment and the food industry. In the built environment, species of section *Aspergillus* are among the primary colonizers of building materials (Flannigan and Miller 2011). Modern heating systems are designed to remove humidity from buildings, creating opportunities for xerophiles to dominate indoor fungal communities. Also of concern is the growth of these fungi in museums or libraries on historic artefacts such as books, carpets or paintings. They also commonly grow on/in leather, dust, softwood, a variety of textiles and even dried specimens in herbaria (Cavka et al. 2010; Micheluz et al. 2015; Pinar et al. 2013; Pinar et al. 2015; Pitt and Hocking 2009; Raper and Fennell 1965; Samson et al. 2010). For the food industry, these species have an economic impact because they can grow on stored grain, cereals or preserved foods with high sugar (i.e. jams, maple syrup) or salt content (i.e. biltong, dried fish) (Pitt and Hocking 2009; Samson et al. 2010).

Xerophily is a common physiological property of many *Aspergillus* species from several subgenera and sections, enabling those species to grow at low water activity (a_w) or equilibrium relative humidity (ERH) (Flannigan and Miller 2011; Pitt 1975). Water activity is a measure of available water in liquid or solid substrates that has a significant effect on which organisms can grow on foods or other matrices, including building materials (Scott 1957). Reducing a_w is widely used in the food industry to reduce spoilage (Pitt and Hocking 2009). For the built environment, however, it is very difficult and often impractical to measure a_w and as a result relative humidity (RH) is often used as a proxy. Because RH measures moisture in air rather than available water in a substrate, it is not considered a reliable indication of whether growth will actually occur on surfaces in the built environment (Flannigan and Miller 2011). A better measure is ERH because it is more representative of available water and is numerically proportional to a_w (Flannigan and Miller 2011; Pitt and Hocking 2009).

Species of section *Aspergillus* produce many extrolites exhibiting a wide range of biological activities (Frisvad and Larsen 2015a; Gomes et al. 2012; Kanokmedhakul et al. 2011; Li et al. 2008a; Li et al. 2008b; Slack et al. 2009; Smetanina et al. 2007). Most notably, compounds from *A. chevalieri* were shown to be active against *Plasmodium falciparum* (malaria), *Mycobacterium tuberculosis* and cancer cell lines (Kanokmedhakul et al. 2011), an antitumor compound was reported from *A. cristatus*

(Almeida et al. 2010), while many compounds are known to be antioxidants. They also produce mycotoxins, especially echinulin, flavoglaucin and physcion, which are toxic to animals (Ali et al. 1989; Bachmann et al. 1979; Cole and Cox 1981; Greco et al. 2015; Nazar et al. 1984; Rabie et al. 1964; Semeniuk et al. 1971; Slack et al. 2009; Vesonder et al. 1988), but toxicity has not been reported in humans. These species are not considered significant human pathogens, because most infections are superficial, with few cases of invasive infections known (de Hoog et al. 2014). Species commonly grow as saprobes on clinical specimens, such as skin and nails (Hubka et al. 2012). The biggest concern to humans, or nuisance, is the growth of these species inside homes, where exposure to spores and fragments, which contains β-(1, 3)-D-glucan, and other metabolites, cause allergies (Green et al. 2006; Slack et al. 2009).

Xerophilic fungi are well studied from a morphological point of view, but much work remains to develop reference sequence data for them. In this paper, we report on the diversity of *Aspergillus* isolated from house dust using media with low a_w that select for the growth of xerophiles. Reference sequences are released for all species, including those received as *Eurotium* from the Canadian Collection of Fungal Cultures and re-identified here. Furthermore, we describe two new species and report on their extrolite production.

Materials and methods

Strains/sampling and isolations

House dust samples were received from various areas in North America. A modified dilution-to-extinction method (Collado et al. 2007) was used to isolate cultures, as described in Visagie et al. (2014a). Modifications included the use of 48-well titre plates rather than 96-well microtube plates and the use of Dichloran 18% Glycerol agar (DG18; (Hocking and Pitt 1980)), Malt extract yeast extract 10% glucose 12% NaCl agar (MY10-12) and Malt extract yeast extract 50% glucose agar (MY50G) (Samson et al. 2010) isolation media to select for xerophilic fungi.

In addition to newly obtained house dust isolates, several strains, including unidentified isolates and some reference or ex-type cultures, of *Aspergillus* sect. *Aspergillus* were obtained from the Canadian Collection of Fungal Cultures, Canada (DAOMC) and the CBS-KNAW Fungal Biodiversity Centre, the Netherlands (CBS).

Morphology

Colony characters were recorded from cultures grown for 7 d on various media, including CYA (Czapek yeast autolysate agar), MEA (Blakeslee's malt extract agar), CREA (Creatine sucrose agar), CY20S (CYA with 20% sucrose agar), MEA20S (MEA with

20% sucrose agar), DG18, YES (yeast extract sucrose agar), M40Y (Harrold's agar; 2% malt, 0.5% yeast extract, 40% sucrose), MY50G and MY10-12 (Harrold 1950; Pitt and Hocking 2009; Samson et al. 2014). Plates were incubated upside down in the dark at 25 °C and left unwrapped. Additional CY20S, DG18 and MEA20S plates were wrapped and incubated at 37 °C. Colour names and codes in descriptions are from Kornerup and Wanscher (1967). Microscopic preparations were made from colonies growing on DG18 and observations made using an Olympus SZX12 dissecting and Olympus BX50 compound microscopes equipped with Infinity3 and InfinityX cameras using Infinity Analyze v. 6.5.1 software (Lumenera Corp., Ottawa, Canada). Variation of conidia and ascospores was evaluated by measuring at least 50 structures and presented as mean +/- standard deviation. Photographic plates were prepared in Pixelmator iOS v. 2.3 (http://www.pixelmator.com/ios), with photomicrographs modified for aesthetic purposes using the repair tool, without altering scientifically significant areas.

DNA extraction, sequencing and phylogenetic analysis

DNA was extracted from 8–10 d old colonies grown on DG18 using the UltracleanTM Microbial DNA isolation Kit (MoBio Laboratories Inc., Solana Beach, USA). Loci chosen for amplification included ITS barcodes (internal transcribed spacer rDNA region, including ITS1-5.8S-ITS2) (Schoch et al. 2012), *BenA* (partial β-tubulin), *CaM* (partial calmodulin) and *RPB2* (RNA polymerase II second largest subunit). Thermocycler programs used for amplification followed Samson et al. (2014) and employed primer pairs V9G & LS266 (ITS; (de Hoog and Gerrits van den Ende 1998; Masclaux et al. 1995), Bt2a & Bt2b (*BenA*; (Glass and Donaldson 1995)), CF1 & CF4 or sometimes CMD5 & CMD6 (*CaM*; (Hong et al. 2006; Peterson et al. 2005)) and 5F & 7CR (*RPB2*; (Liu et al. 1999)). Sequencing was done as described in Visagie et al. (2016). Contigs were assembled in Geneious v. 8.1.8 (Biomatters Ltd, New Zealand) and newly generated sequences submitted to GenBank.

As a preliminary step in identification, *CaM* sequences derived from the newly isolated cultures were compared to an ex-type reference sequence database published by Samson et al. (2014). Then, gene sequences of the two presumed to be new sect. *Aspergillus* species were compared to reference datasets obtained from Peterson (2008), Hubka et al. (2013) and Visagie et al. (2014a). All datasets were aligned in MAFFT v. 7.221 (Katoh and Standley 2013) using the L-INS-i option for ITS and G-INS-i option for the other genes. All alignments were trimmed in Geneious and then analysed as single and concatenated datasets using Maximum Parsimony (MP) and Bayesian Inference of phylogenetic trees (BI). For concatenated phylogenies, a partitioned dataset of ITS, *BenA*, *CaM* and *RPB2* regions was used.

MP analyses were run in PAUP* v. 4.0b10 (Swofford 2002) using heuristic searches with 100 random taxon additions and gaps treated as missing data. Support in nodes was calculated using a bootstrap analysis with the heuristic search option and 1000 replicates.

BI analyses were run in MrBayes v. 3.2.5 (Ronquist et al. 2012). Model selections for BI were made for each gene based on the lowest Akaike Information Criterion (AIC) value, calculated in MrModeltest v. 2.3 (Nylander 2004). Analyses were run with two sets of four chains and stopped at a split frequency of 0.01. The sample frequency was set at 100 and 25 percent of trees removed as burnin. Trees were visualized in FigTree v. 1.4.2 (http://tree.bio.ed.ac.uk/software/figtree/) and prepared for publication in Adobe® Illustrator® CS6. Aligned datasets, command blocks and trees were uploaded to TreeBase (www.treebase.org) under submission number 19771.

Extrolite analysis

For extrolite analysis, all strains were grown on 9 cm polystyrene Petri dishes on MEA supplemented with 7.5% NaCl at 25 °C for 14 d. Six agar plugs from each fungal isolate were removed with a sterilized 7 mm cork borer and placed into a 13 mL polypropylene tube. Ethyl acetate (2 mL) was added to the tubes and vortexed for 30 s, followed by 1 h of sonication at 30 °C and vortexed again for 30 s. The supernatants were transferred into clean polypropylene tubes and dried on a centrifugal vacuum concentrator at 35° C. Extracts were reconstituted in 1 mL of 8:2 methanol:water and filtered into 2 mL amber glass HPLC vials using a 0.45 µm PVDF syringe filter. Extracts were immediately stored at -20 °C until LC-MS analysis. Extracts were analyzed on a Q-Exactive orbitrap coupled to a 1290 Agilent HPLC in both positive and negative polarities. Chemical formula of observed extrolites were determined with Xcalibur® software using accurate mass measurements and manually verified by isotopic pattern. Chemical formulae were searched against AntiBase2013 and Scifinder and putatively confirmed by comparing product ions observed with those published in the literature or though manual interpretation. The fungi were also analysed using the HPLC-DAD method described by Frisvad and Thrane (1987) as modified by Nielsen et al. (2011), by taking two agar plugs from each of the following media: DG18, CYA20S and YES agar, and extracting the combined 6 agar plugs of the colonies of *Aspergillus* with ethylacetate / isopropanol (3:1, vol./vol.) with 1% (vol.) formic acid added to that mixture. The retention indices and UV spectra were compared to those given in the supplementary material of the Nielsen et al. (2011) paper.

Results

Sampling, isolations and identification

Isolations from house dust collected in Canada and Hawaii resulted in 1039 isolates of xerophilic/xerotolerant fungi. 296 isolates were identified as *Aspergillus*, of which members from sections *Aspergillus*, *Nidulantes* (*A. versicolor* clade) and *Restricti* were most

abundant. Strains were identified to species using *CaM* sequences and identities confirmed by morphological examination. They include *A. chevalieri*, *A. cibarius*, *A. montevidensis*, *A. proliferans*, *A. pseudoglaucus*, *A. ruber* and *A. tonophilus* from sect. *Aspergillus*. In section *Nidulantes* (*A. versicolor* clade), *A. jensenii* and *A. sydowii* were isolated most frequently, while *A. creber*, *A. fructus*, *A. protuberus*, *A. tenneseensis* and *A. versicolor* were also recovered. A large degree of sequence diversity was observed in sect. *Restricti* and will be presented in a separate study. Other *Aspergillus* species identified include *A. aureolatus*, *A. candidus*, *A. calidoustus*, *A. flavus*, *A. japonicus*, *A. lentulus*, *A. luchuensis*, *A. micronesiensis*, *A. niger*, *A. pragensis*, *A. tamarii*, *A. terreus*, *A. tubingensis*, *A. welwitschiae* and *A. westerdijkiae.* Reference sequences, mostly *CaM*, obtained for these species were uploaded to GenBank under accession numbers KX894565–KX894666 and KY351765–KY351785, and are included in Suppl. material 1: Table 1 to assist with future identifications. This table also include additional information with regard to strains' location and growth medium used for their isolations. During this survey, two sect. *Aspergillus* species with eurotium-like sexual states could not be identified as known species and are described below as new species, based on growth characters on a wide range of culture media. The new species are compared with their close relatives and notes are provided on their diagnostic phenotypic characters, including extrolite production.

Phylogeny

To demonstrate genealogical concordance for the two new species, phylogenies for all known species of sect. *Aspergillus* were prepared (Table 1) using alignments of ITS, *BenA*, *CaM*, and *RPB2* (Fig. 1) and overall phylogenetic relationships considered as a concatenated dataset (Fig. 2).

The ITS alignment was 535 bp long and contained 68 variable characters, of which 27 were parsimony informative. MP analysis resulted in two equally parsimonious trees (length 79 steps, CI = 0.987, RI = 0.992). HKY+I was found to be the most suitable model for BI analysis. ITS is highly conserved in sect. *Aspergillus*, as demonstrated in the phylogenetic analysis, making it uninformative as an identification barcode in section *Aspergillus*. Of the 22 species, including the two new species described here, only *A. cumulatus*, *A. leucocarpus*, *A. osmophilus* and *A. xerophilus* have unique ITS barcodes. The alignments for the *BenA*, *CaM* and *RPB2* datasets were respectively 389 (151 variable, 136 parsimony informative), 556 (221 variable, 177 parsimony informative) and 871 bp (202 variable, 162 parsimony informative) long. MP analyses resulted in 84 (length 287 steps, CI = 0.728, RI = 0.923), 12 (length 275 steps, CI = 0.7, RI = 0.904), 28 (length 364 steps, CI = 0.648, RI = 0.911) and 24 (length 798 steps, CI = 0.692, RI = 0.907) equally parsimonious trees for *BenA*, *CaM*, *RPB2* and concatenated dataset. K80+G (*BenA*), SYM+G (*CaM*) and SYM+I+G (*RPB2*) were the most suitable models for BI.

Tree topologies did not differ for respective genes between MP and BI; therefore, MP trees were used to present results. Some species are consistently resolved as sister

Table 1. Strains used for phylogenetic analyses.

			GenBank accession numbers			
Species	**Strains**	**Origin**	**ITS**	**CaM**	**BenA**	**RPB2**
Aspergillus appendiculatus	CBS 374.75T; DAOMC 231665; IMI 278374; ETH 8286	Smoked sausage, Switserland	HE615132	HE801318	HE801333	HE801307
Aspergillus appendiculatus	CBS101746; AS 3.4673	Sheep dung, China	HE615133	HE801319	HE801334	HE801308
Aspergillus brunneus	CBS 112.26T; NRRL131; ATCC 1021; IMI 211378; MUCL 15646	Fig, USA	EF652060	EF651998	EF651907	EF651939
Aspergillus brunneus	CBS 113.27; NRRL124; ATCC 1036; IMI 029188	Unknown	EF652056	EF651997	EF651904	EF651938
Aspergillus brunneus	NRRL 133	Unknown	EF652061	EF651999	EF651908	EF651940
Aspergillus chevalieri	CBS 522.65T; NRRL 78; ATCC 16443; IMI 211382	Coffee beans, USA	EF652068	EF652002	EF651911	EF651954
Aspergillus chevalieri	NRRL 4755	Contaminated culture, USA	EF652071	EF652004	EF651913	EF651956
Aspergillus chevalieri	NRRL 79	Unknown, USA	EF652069	EF652003	EF651912	EF651955
Aspergillus cibarius	KACC 46346T	Meju, Korea	JQ918177	JQ918183	JQ918180	JQ918186
Aspergillus cibarius	KACC 46764	Meju, Korea	JQ918178	JQ918184	JQ918181	JQ918187
Aspergillus cibarius	KACC 46765	Meju, Korea	JQ918179	JQ918185	JQ918182	JQ918188
Aspergillus costiformis	CBS 101749T; AS 3.4664	Rotten paper, China	HE615136	HE801320	HE801338	HE801309
Aspergillus cristatus	CBS 123.53T; NRRL 4222; ATCC 16468; IMI 172280; MUCL 15644	Unknown, South Africa	EF652078	EF652001	EF651914	EF651957
Aspergillus cumulatus	KACC 47316T	Rice straw, Korea	KF928303	KF928300	KF928297	KF928294
Aspergillus cumulatus	KACC 47513	Indoor air from meju fermentation room, Korea	KF928304	KF928301	KF928298	KF928295
Aspergillus cumulatus	KACC 47514	Indoor air from meju fermentation room, Korea	KF928305	KF928302	KF928299	KF928296
Aspergillus glaucus	CBS 516.65T; NRRL 116; ATCC 16469; IMI 211383	Unpainted basement board, USA	EF652052	EF651989	EF651887	EF651934
Aspergillus glaucus	NRRL 117; ATCC 66470	Unpainted basement board, USA	EF652053	EF651990	EF651888	EF651935
Aspergillus glaucus	NRRL 120; ATCC 16925; FRR 120	Unknown	EF652054	EF651991	EF651889	EF651936
Aspergillus glaucus	NRRL 121; IMI 313756	Unknown	EF652055	EF651992	EF651890	EF651937
Aspergillus intermedius	CBS 377.75; IMI 278376; ETH 8277	Soil, Spain	HE974459	HE974437	HE974432	HE974425
Aspergillus intermedius	CBS 523.65T; NRRL 82; ATCC 16444; IMI 089278; IMI 089278ii; DSM 2830	Unknown, United Kingdom	EF652074	EF652012	EF651892	EF651958

			GenBank accession numbers			
Species	**Strains**	**Origin**	**ITS**	**CaM**	**BenA**	**RPB2**
Aspergillus intermedius	NRRL 4817; IMI 313754	Unknown	EF652072	EF652014	EF651894	EF651960
Aspergillus intermedius	NRRL 84	Unknown	EF652070	EF652013	EF651893	EF651959
Aspergillus leucocarpus	CBS 353.68T; NRRL3497; IMI 278375	Raw sausage, Germany	EF652087	EF652023	EF651925	EF651972
Aspergillus mallochii	DAOMC 146054T = CBS 141928 = DTO 357A5 = KAS 7618	Pack rat dung, USA	KX450907	KX450902	KX450889	KX450894
Aspergillus mallochii	CBS 141776 = DTO 343G3	'*Chocolat miroir*' icing for cake, the Netherlands	KX450908	KX450903	KX450890	KX450895
Aspergillus megasporus	DAOMC 250799T = CBS 141929= DTO 356H7 = KAS 6176	House dust, Canada	KX450910	KX450905	KX450892	KX450897
Aspergillus megasporus	DAOMC 250800 = DTO 356H1 = KAS 5973	House dust, Canada	KX450909	KX450904	KX450891	KX450896
Aspergillus megasporus	CBS 141772 = DTO 048I3	Dutch chocolate butter, the Netherlands	KX450911	KX450906	KX450893	KX450898
Aspergillus montevidensis	CBS 491.65T; NRRL 108; ATCC 10077; IMI 172290; IHEM 3337	Human tympanic membrane, unknown	EF652077	EF652020	EF651898	EF651964
Aspergillus montevidensis	CBS 518.65; NRRL90; ATCC 16464; IMI 229971; IFO 33018	Unknown, USA	EF652076	EF652017	EF651897	EF651963
Aspergillus montevidensis	NRRL 4716; IMI 350348	Candied grapefruit rind, USA	EF652079	EF652018	EF651899	EF651965
Aspergillus montevidensis	NRRL 89; ATCC 10065; IMI 211806	Unknown	EF652075	EF652016	EF651896	EF651962
Aspergillus neocarnoyi	CBS 471.65T; NRRL126; ATCC 16924; IMI 172279	Unknown	EF652057	EF651985	EF651903	EF651942
Aspergillus niveoglaucus	CBS 101750; AS 3.4665	Soil, China	HE615135	HE801323	HE801331	HE801312
Aspergillus niveoglaucus	CBS 114.27T; NRRL127; NRRL 129; NRRL 130; ATCC 10075; CBS 517.65; IMI 032050; IMI 032050ii	Unknown	EF652058	EF651993	EF651905	EF651943
Aspergillus niveoglaucus	NRRL 128; FRR 128; IMI 091871	Unknown	EF652059	EF651994	EF651906	EF651944
Aspergillus niveoglaucus	NRRL 136	Unknown	EF652062	EF651995	EF651909	EF651945
Aspergillus niveoglaucus	NRRL 137; IMI 091872	Unknown	EF652063	EF651996	EF651910	EF651946
Aspergillus osmophilus	CBS 134258T; IRAN 2090C	Leaf of *Triticum aestivu*, Iran	KC473921	KC473918	KC473924	KX512310
Aspergillus proliferans	CBS 121.45T; NRRL 1908; CBS 528.65; ATCC 16922; IMI 016105; IMI 016105ii; IMI 016105iii; MUCL 15625	Cotton yarn, United Kingdom	EF652064	EF651988	EF651891	EF651941
Aspergillus proliferans	NRRL 114; ATCC 10076; IMI 211808	Unknown, USA	EF652051	EF651987	EF651886	EF651933
Aspergillus proliferans	NRRL 62482; CCF 4096	Palm skin, Czech Republic	FR848827	HE650908	FR775375	HE801303
Aspergillus proliferans	NRRL 62494; CCF 4146	Toenail, Czech Republic	HE578067	HE650909	HE578076	HE801304
Aspergillus proliferans	NRRL 62497; CCF 4115	Toenail, Czech Republic	FR851850	HE578090	FR851855	HE578107

			GenBank accession numbers			
Species	**Strains**	**Origin**	**ITS**	**CaM**	**BenA**	**RPB2**
Aspergillus proliferans	NRRL 71	Leafhoppers, USA	EF652047	EF651986	EF651885	EF651932
Aspergillus pseudoglaucus	CBS 123.28T; NRRL 40; ATCC 10066; IMI 016122; IMI 016122ii; MUCL 15624	Unknown	EF652050	EF652007	EF651917	EF651952
Aspergillus pseudoglaucus	CBS 379.75; IMI 278373; ETH 8218; DSM 1370	Leaf from *Vaccinium myrtillus*, Switserland	HE615131	HE801322	HE801336	HE801311
Aspergillus pseudoglaucus	CBS 529.65; NRRL13; NRRL 24; ATCC 9294; IMI 016114; IMI 016114ii; MUCL 15649	Prunus domestica, France	EF652048	EF652005	EF651915	EF651950
Aspergillus pseudoglaucus	CBS101747; AS 3.4674	Animal dung, China	HE615130	HE801321	HE801335	HE801310
Aspergillus pseudoglaucus	NRRL 17; ATCC 10079; UAMH 6580	Skin from wrist, USA	EF652049	EF652006	EF651916	EF651951
Aspergillus ruber	CBS 101748; AS 3.4632	Soil, China	HE615134	HE801325	HE801337	HE801315
Aspergillus ruber	CBS 464.65; NRRL5000; ATCC 16923; IMI 32048	Coffee beans, United Kingdom	EF652080	EF652010	EF651922	EF651949
Aspergillus ruber	CBS 530.65T; NRRL 52; ATCC 16441; IMI 211380	Unknown	EF652066	EF652009	EF651920	EF651947
Aspergillus ruber	NRRL 76; IMI 91868	Unknown	EF652067	EF652011	EF651921	EF651948
Aspergillus sloanii	CBS 138176; DTO 244-I8	House dust, United Kingdom	KJ775539	KJ775308	KJ775073	KX463364
Aspergillus sloanii	CBS 138177T; DTO 245-A1	House dust, United Kingdom	KJ775540	KJ775309	KJ775074	KX463365
Aspergillus sloanii	CBS 138178; DTO 245-A8	House dust, United Kingdom	KJ775542	KJ775313	KJ775076	KX450900
Aspergillus sloanii	CBS 138179; DTO 245-A9	House dust, United Kingdom	KJ775543	KJ775314	KJ775077	KX450901
Aspergillus sloanii	CBS 138231; DTO 245-A6	House dust, United Kingdom	KJ775541	KJ775311	KJ775075	KX450899
Aspergillus tonophilus	CBS 405.65T; NRRL 5124; ATCC 14567; ATCC 16440; ATCC 36504; DSM 3462; IFO 6529; IMI 108299; IMI 108299ii	Binocular lens, Japan	EF652081	EF652000	EF651919	EF651969
Aspergillus xerophilus	CBS 938.73T; NRRL6131; FRR 2804; IMI 278377	Desert soil, Egypt	EF652085	EF651983	EF651923	EF651970
Aspergillus xerophilus	NRRL 6132	Desert soil, Egypt	EF652086	EF651984	EF651924	EF651971

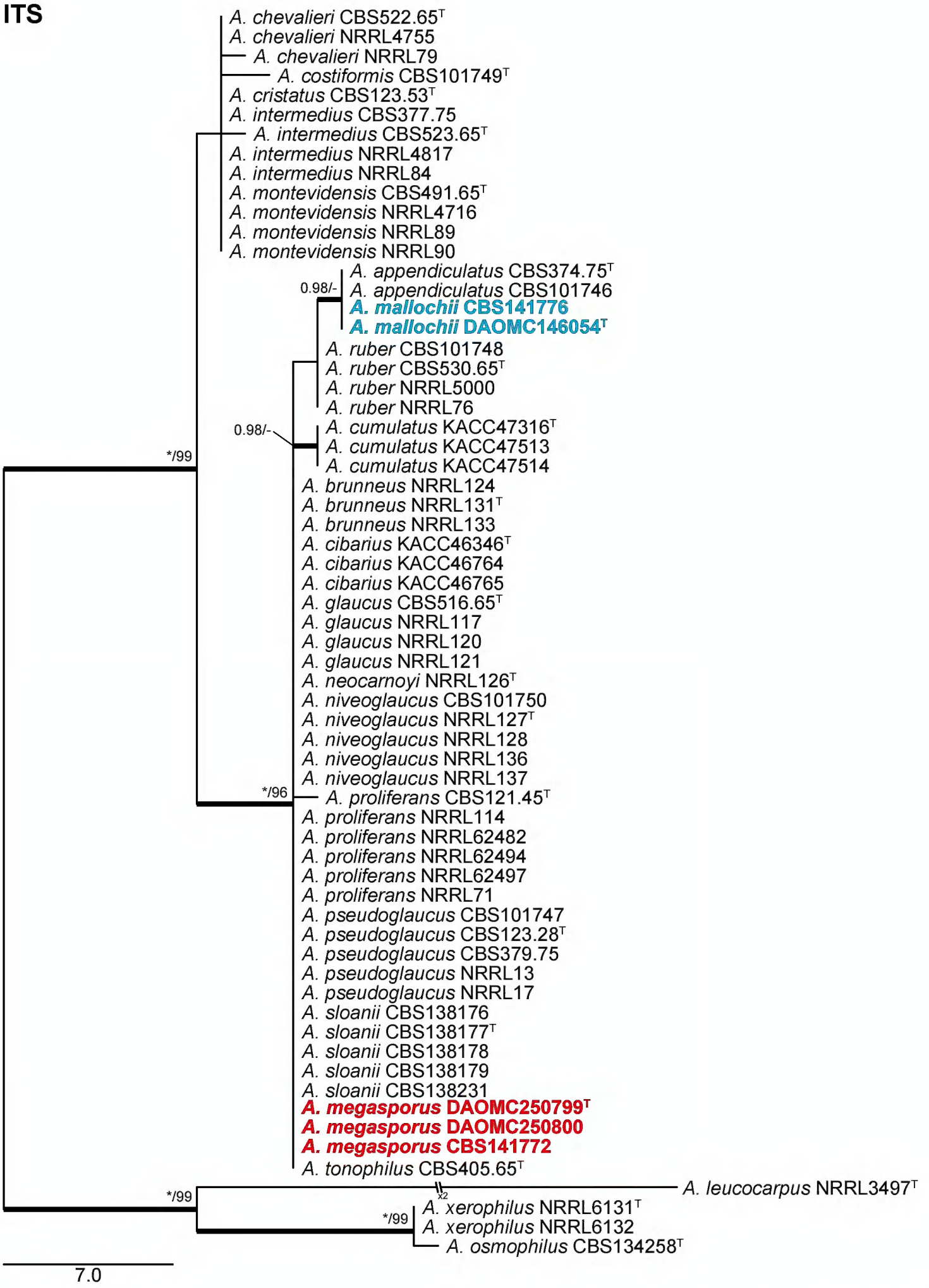


Figure 1. One of the most parsimonious trees of *Aspergillus* sect. *Aspergillus* based on ITS, *CaM*, *BenA* and *RPB2*. Trees were rooted to *A. xerophilus*, *A. leucocarpus* and *A. osmophilus*. Support in nodes higher than 80% bootstrap values and 0.95 posterior probabilities are shown above thickened branches. New species are shown in bold and colour, while ex-type strains are followed by T.

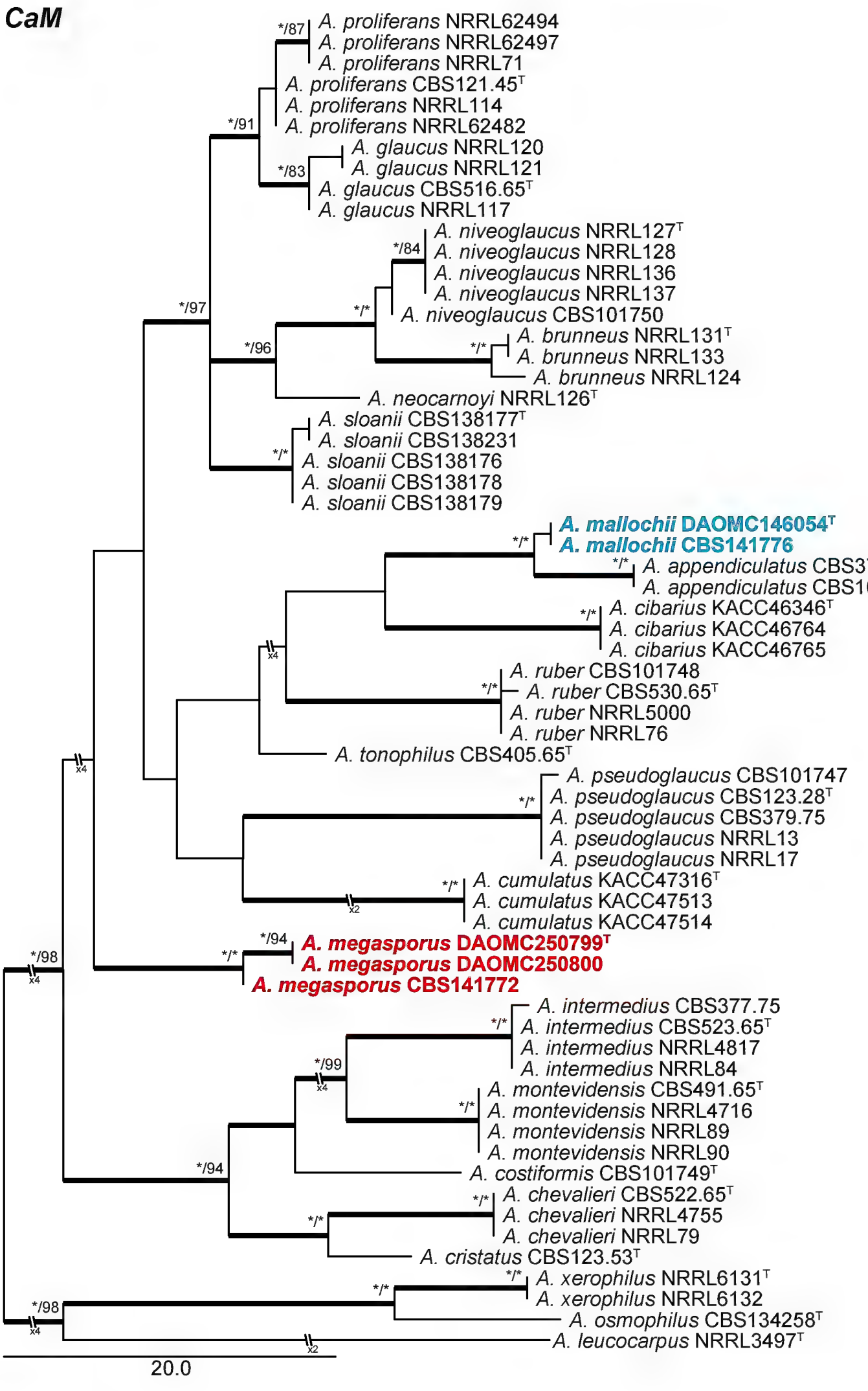


Figure 1. Continued.

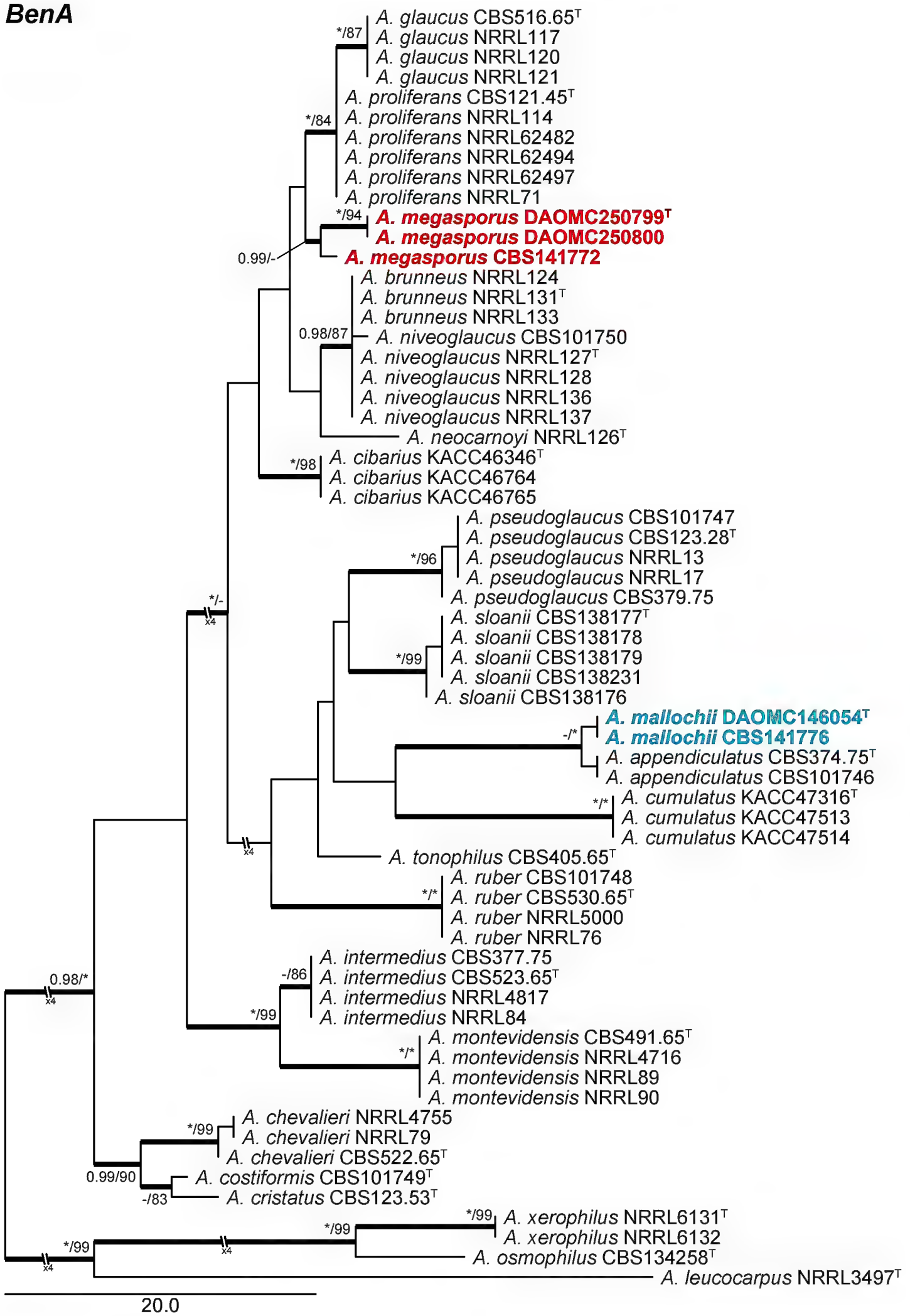


Figure 1. Continued.

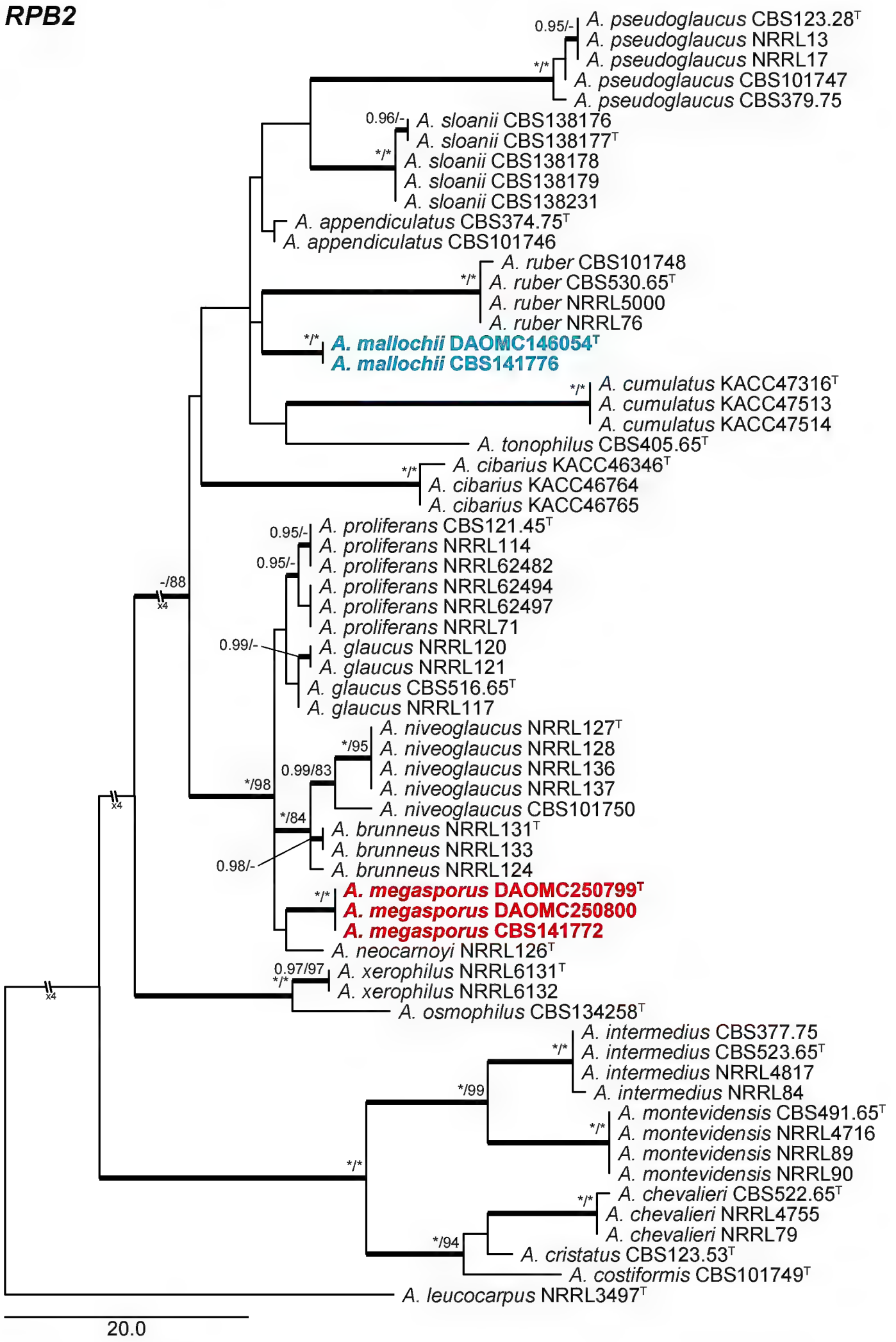


Figure 1. Continued.

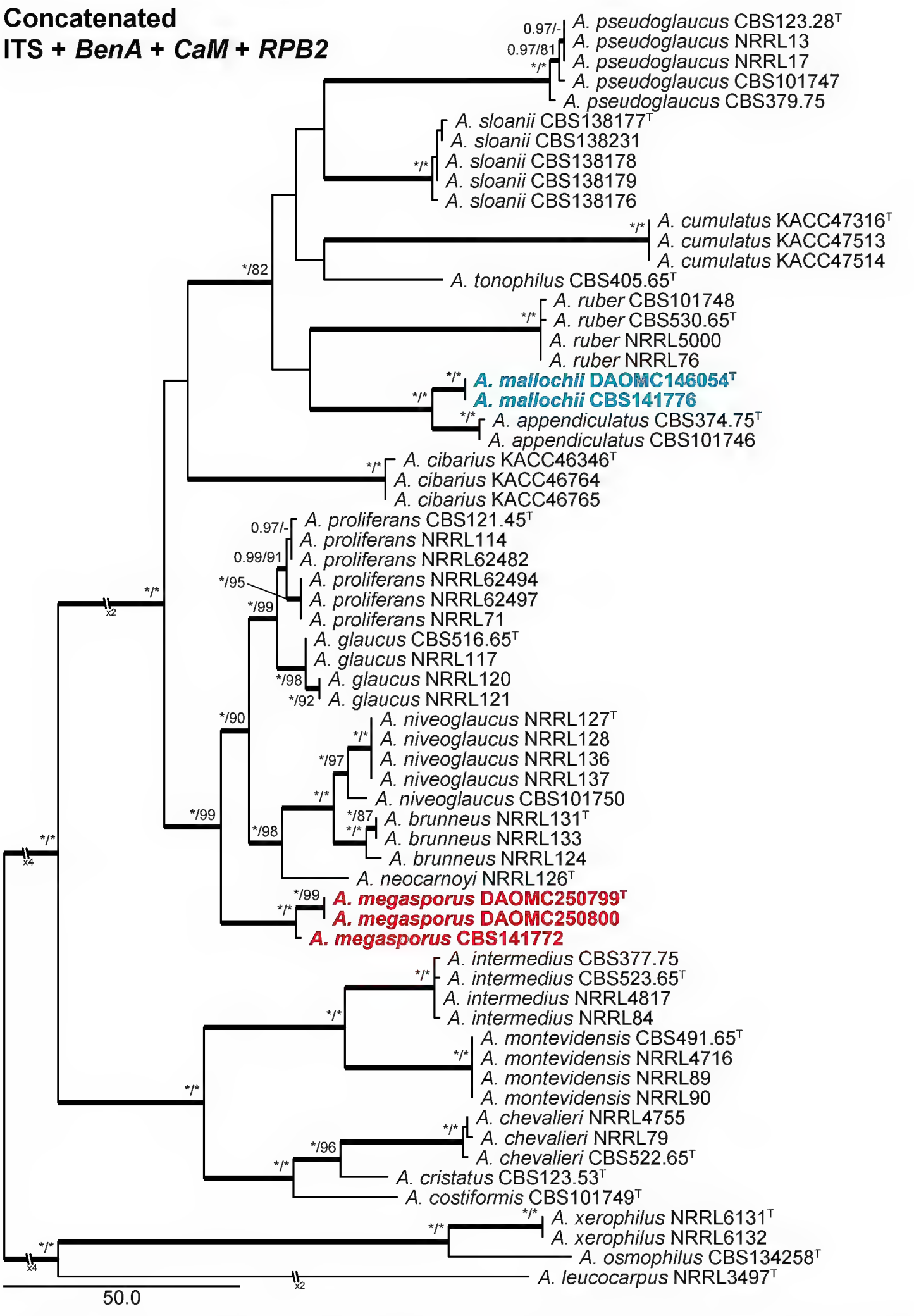


Figure 2. One of the most parsimonious trees of *Aspergillus* sect. *Aspergillus* based on a combined dataset of ITS, *BenA*, *CaM* and *RPB2*. The tree was rooted to *A. xerophilus*, *A. leucocarpus* and *A. osmophilus*. Support in nodes higher than 80% bootstrap values and 0.95 posterior probabilities are shown above thickened branches. New species are shown in bold and colour, while ex-type strains are followed by T.

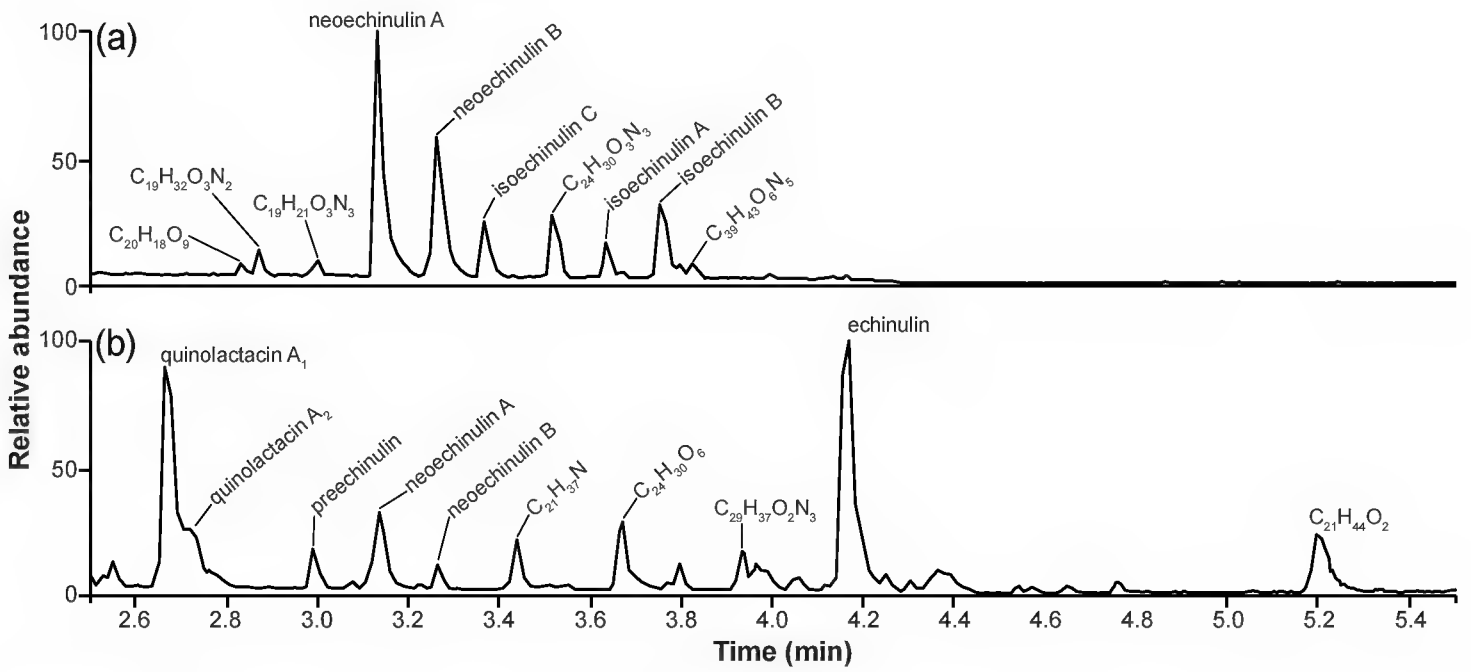
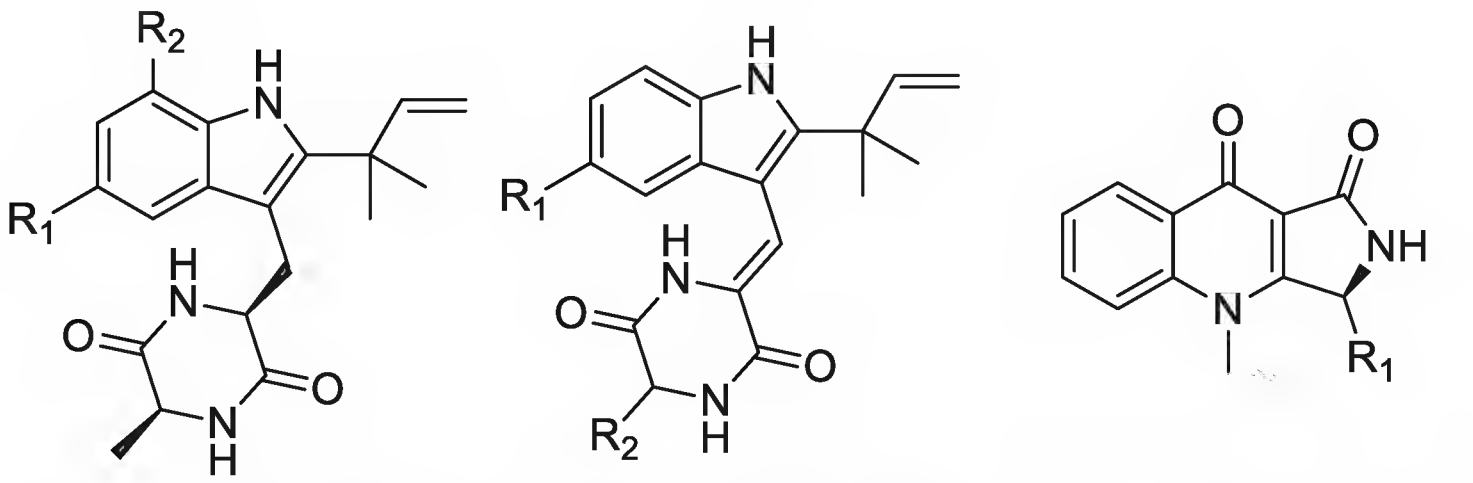


Figure 3. Base peak chromatograms observed in positive ionization mode. **a** *Aspergillus mallochii* (DAOMC 146054 = KAS 7618) **b** *Aspergillus megasporus* (DAOMC 250799 = KAS 6176). Both species show some production of echinulin class of alkaloids to varying amounts. Quinolactacin A1, A2 and B were not detected in *A. mallochii*.

species such as *A. proliferans* and *A. glaucus*, *A. brunneus* and *A. niveoglaucus*, *A. montevidensis* and *A. intermedius*, and *A. osmophilus* and *A. xerophilus.* On a deeper level, however, the backbones in all gene trees were generally poorly supported, resulting in inconsistent clades among different gene trees. The addition of more newly discovered species of section *Aspergillus* in future may result in better backbone support. With regards to the new species, *A. mallochii* was sister to *A. appendiculatus*, although *RPB2* placed it on a unique branch. *Aspergillus megasporus* resolves in different positions depending on gene analyzed, but based on the concatenated phylogeny belongs in a clade with *A. brunneus*, *A. niveoglaucus*, *A. neocarnoyi*, *A. glaucus* and *A. proliferans*. For species identifications, it is clear that all three of these genes are superior to ITS and distinguish between all 22 accepted species in sect. *Aspergillus*.

Extrolites

Aspergillus mallochii and *A. megasporus* produced several related tryptophan derived alkaloids including, echinulins, neoechinulins and isoechinulins, but in varying amounts (Table 2). *Aspergillus mallochii* (DAOMC 146054) was a major producer of neoechinulin A & B, while also producing isoechinulin A, B & C (Fig. 3a). Quinolactacin A_1, A_2 & B were among the major extrolites produced by *A. megasporus* (Fig. 3b). The other was echinulin produced by DAOMC 250799, although it was not detected in DAOMC 250800. The latter strain was generally a poor extrolite producer. The chemical structures of major extrolites produced by *A. megasporus* and *A. mallochii* are shown in Fig. 4.



	R_1	R_2
echinulin	C_5H_9	C_5H_9
preechinulin	H	H

	R_1	R_2
isoechinulin A	C_5H_9	CH_3
isoechinulin B	C_5H_9	CH_2
isoechinulin C	C_5H_9O	CH_2
neoechinulin A	H	CH_3
neoechinulin B	H	CH_2

	R_1
quinolactacin A_1	(R) sec-butyl
quinolactacin A_2	(S) sec-butyl
quinolactacin B	i-propyl

Figure 4. Chemical structure of major compounds produced by *A. mallochii* and *A. megasporus*.

Table 2. Overview of the major extrolites detected and product ions.

Extrolite	*m/z*	Formula	RT (min)	Product ions *m/z*				
echinulin	462,311	$C_{29}H_{39}N_3O_2$	4,16	338,186	266,190	198,128	210,128	270,124
isoechinulin A	392,233	$C_{24}H_{29}N_3O_2$	3,63	268,108	336,170	256,108	69,071	–
isoechinulin B	390,217	$C_{24}H_{27}O_2N_3$	3,75	266,092	322,155	334,155	254,092	306,123
isoechinulin C	406,212	$C_{24}H_{27}O_3N_3$	3,37	334,155	266,092	237,138	338,150	–
neoechinulin A	324,171	$C_{19}H_{21}O_2N_3$	3,14	256,108	268,108	185,071	69,071	–
neoechinulin B	322,155	$C_{19}H_{19}O_2N_3$	3,26	254,092	266,095	69,071	226,097	–
preechinulin	326,186	$C_{19}H_{23}O_2N_3$	2,99	130,065	198,128	270,123	258,124	–
quinolactacin A1	271,144	$C_{16}H_{18}N_2O_2$	2,66	214,073	–	–	–	–
quinolactacin A2	271,144	$C_{16}H_{18}N_2O_2$	2,72	214,073	–	–	–	–
quinolactacin B	257,129	$C_{15}H_{16}N_2O_2$	2,54	214,073	–	–	–	–
questin*	283,061	$C_{16}H_{12}O_5$	3,39	268,038	240,042	–	–	–

* observed in negative ionization mode

Taxonomy

Aspergillus mallochii **Visagie, Yilmaz & Seifert, sp. nov.**
MycoBank MB 819025
Fig. 5

Etymology. Latin, *mallochii*, named after Prof. David Malloch, a Canadian specialist in 'Plectomycetes' who first collected this species in the 1960's.

Typus. USA, California, San Mateo, pack rat dung, added to DAOMC in 1969, collected by David Malloch, Holotype DAOM 740296, culture ex-type DAOMC 146054 = CBS 141928 = DTO 357-A5 = KAS 7618.

Additional material examined. The Netherlands, 'chocolat miroir' icing for a cake, unknown date and collector, culture CBS 141776 = DTO 343-G3.

ITS barcode. KX450907. Alternative identification markers: *BenA* = KX540889, *CaM* = KX450902, *RPB2* = KX450894.

Colony diam, 7 d (in mm), 25 °C. CYA 6–8; CY20S 14–17; MEA 3–4; MEA20S 29–31; DG18 48–50; YES 9–10; M40Y 48–50; MY50G 35–40; MY10-12 29–30; CY20S, DG18, MEA20S at 37 °C no growth; CREA no growth.

Colony characters. CYA: Colonies with restricted growth; conidiophores sparse; cleistothecia absent. CY20S: Colonies grow faster than on CYA; sporulation sparse to moderately dense, greyish to dark green (30E5–F5); cleistothecia dark yellow, abundant at colony centre. MEA: Colonies with restricted growth; conidiophores and cleistothecia absent. MEA20S: Colonies grow faster than on MEA; sporulation sparse, greyish to dark green (30E5–F5); cleistothecia yellow to orange, abundant. DG18: Colonies very fluffy with aerial mycelia giving rise to conidiophores; sporulation sparse to moderately dense, greyish to dark green (30E5–F5); cleistothecia abundant at colony centre, yellow to orange. Homothallic.

Micromorphology on DG18. Cleistothecia eurotium-like, wall consisting of one layer of flattened cells, yellow to orange, turning deep brown with age, globose, 95–250 µm diam. Asci eight-spored, globose, ellipsoidal to pyriform, 10–15 µm diam, maturing after 7–14 d. Ascospores lenticular, equatorial crest present but incomplete, convex surface roughened, 4.5–6 × 3.5–4.5 µm (5.1±0.3 × 3.9±0.3), n = 52. Conidiophores radiate and columnar, uniseriate; stipes smooth, 200–1000 × 7.5–17(–19) µm; vesicle globose, (25–)40–65 µm diam; phialides ampulliform, covering 80–100% of vesicle, 7–11 × 3–5 µm; conidia roughened to spiny, ellipsoidal, connectives easily visible, 4.5–6.5 × 4–5.5 µm (5.4±0.4 × 4.5±0.3), average width/length = 0.83, n = 68.

Extrolites. Isoechinulin A, B & C; neoechinulin A & B; unknowns $C_{20}H_{18}O_9$, $C_{19}H_{32}O_3N_2$, $C_{19}H_{21}O_3N_3$, $C_{24}H_{30}O_3N_3$, $C_{39}H_{43}O_6N_5$. Additionally, echinulin, erythroglaucin, auroglaucin, flavoglaucin, dihydroauroglaucin, tetrahydroauroglaucin were found in CBS 141776. Some extrolites tentatively identified as tetracyclic compounds were detected in CBS 141776.

Notes. *Aspergillus mallochii* is phylogenetically and morphologically most similar to *A. appendiculatus.* Both are unable to grow at 37 °C and both have ascospores with incomplete equatorial furrows. Ascospores of the new species, however, are generally smaller and at least finely roughened compared to the smoother ascospores of *A. appendiculatus*.

Aspergillus megasporus **Visagie, Yilmaz & Seifert, sp. nov.**
MycoBank MB 819028
Fig. 6

Etymology. Latin, *megasporus*, in reference to the large conidia produced by this species.

Figure 5. *Aspergillus mallochii* (DAOMC 146054). **a** Colonies on MEA, MEA20S, MY10-12 (top row, from left to right), DG18, CY20S, MY50G (bottom row, from left to right) **b** Texture on DG18 **c** Asci **d** Ascospores **e** Cleistothecium **f, g** Conidiophores **h** Conidia. Scale bars: **e** = 50 µm, **c, d, f–h** = 10 µm.

Figure 6. *Aspergillus megasporus* (DAOMC 250799). **a** Colonies on MEA, MEA20S, MY10-12 (top row, from left to right), DG18, CY20S, MY50G (bottom row, from left to right) **b** Texture on DG18 **c** Asci **d** Ascospores **e** Cleistothecium **f, g** Conidiophores **h** Conidia. Scale bars: **e** = 50 µm, **c, d, f–h** = 10 µm.

Typus. Canada, Nova Scotia, Wolfville, house dust, 29 January 2015, collected by Allison Walker, isolated by Cobus M. Visagie, holotype DAOM 741781, culture ex-type DAOMC 250799 = CBS 141929= DTO 356-H7 = KAS 6176.

Additional material examined. Canada, New Brunswick, Little Lepreau, house dust, 29 January 2015, collected by Allison Walker, isolated by Cobus M. Visagie, culture DAOMC 250800 = DTO 356-H1 = KAS 5973. **The Netherlands**, Dutch chocolate butter, August 2007, collected and isolated by Martin Meijer, culture CBS 141772 = DTO 048-I3.

ITS barcode. KX540910. Alternative identification markers: *BenA* = KX450892, *CaM* = KX450905, *RPB2* = KX450897.

Colony diam, 7 d (in mm), 25 °C. CYA 3–8; CY20S 30–35; MEA 3–5; MEA20S 24–35; DG18 47–50; YES 15–16; M40Y 45–47; MY50G 35–40; MY10-12 40–44; CY20S, DG18, MEA20S at 37 °C no growth, CREA no growth.

Colony characters. CYA: Colonies with restricted growth; conidiophores and cleistothecia absent. CY20S: Colonies grow faster than on CYA; sporulation moderately dense, greyish to dark green (30E5–F5); cleistothecia yellow, sparse. MEA: Colonies with restricted growth; conidiophores and cleistothecia absent. MEA20S: Colonies grow faster than on MEA; sporulation moderately dense, greyish to dark green (30E5–F5); cleistothecia yellow, moderately abundant. DG18: Colonies very fluffy with abundant aerial mycelia giving rise to conidiophores; sporulation moderately dense, dull to dark green (28E3–F3); cleistothecia abundant, dark yellow to orange. Homothallic.

Micromorphology on DG18. Cleistothecia eurotium-like, wall consisting of one layer of flattened cells, yellow to orange, globose, 115–205 µm diam. Asci eight-spored, globose, ellipsoidal to pyriform, 14–19.5 µm diam. Ascospores lenticular, equatorial crest roughened, convex surface smooth, 5–8 × 3.5–6 µm (6.4±0.6 × 4.9±0.5), n = 51. Conidiophores radiate and columnar, uniseriate; stipes smooth, (30–)60–1000 × (9–)13–20 µm; vesicle globose, (8.5–)20–60 µm diam; phialides ampulliform, covering 70–100% of vesicle, (9–)11–15 × 5–7 µm; conidia roughened to spiny, ellipsoidal, connectives often visible, 7–12 × 6–8.5 µm (9.5±1.0 × 6.9±0.5), average width/length = 0.72, n = 85.

Extrolites. Echinulin; neoechinulin A & B; preechinulin; quinolactacin A_1 & A_2; unknowns $C_{15}H_{20}O_2$, $C_{21}H_{37}N$, $C_{24}H_{30}O_6$, $C_{29}H_{37}O_2N_3$, $C_{21}H_{44}O_2$. In addition, asperflavin, emodin, erythroglaucin, physcion and bisanthron were found in CBS 141772. Some additional extrolites, tentatively identified as tetracyclic compounds, were detected in CBS 141772

Notes. The concatenated phylogeny of *BenA*, *CaM* and *RPB2* resolves *A. megasporus* in a clade with *A. brunneus*, *A. niveoglaucus*, *A. neocarnoyi*, *A. glaucus* and *A. proliferans*. None of these species are able to grow on CY20S at 37 °C. *Aspergillus niveoglaucus* and *A. megasporus* can be distinguished from other species by their large conidia, which are up to 11 and 12 µm in the longest axis respectively. *Aspergillus megasporus* colonies grow faster than *A. niveoglaucus* on DG18.

Discussion

Species of *Aspergillus* section *Aspergillus* are xerophilic and widespread in nature. Indoor environments, including homes and public buildings, are designed to be as dry as possible, especially in temperate countries, and these conditions select for these xerophiles to thrive. This partially explains the dominance of *Aspergillus*, *Penicillium*, *Cladosporium* and *Wallemia* in indoor fungal communities (Amend et al. 2010; Flannigan and Miller 2011; Samson et al. 2010; Visagie et al. 2014a). In our isolations of xerophiles occurring in Canadian and Hawaiian house dust, these genera were also found to be dominant. Xerophily is spread broadly across *Aspergillus*. Thirty *Aspergillus* species were isolated in our survey, excluding the many section *Restricti* species that will be addressed in another study. All *Aspergillus* are capable of growth on DG18, but MY10-12 and MY50G have much lower water activities. Species isolated from these selective media included species of sections *Aspergillus*, *Candidi*, *Flavipedes*, *Nidulantes*, *Nigri*, *Restricti* and *Versicolores* (summarized in Suppl. material 1: Table 1). One new species was discovered from our house dust samples, described as *A. megasporus*. We also re-identified all cultures from DAOMC deposited as *Eurotium*. Among these, we discovered an additional species that we described as *A. mallochii* using morphology, extrolite and phylogenetic analyses.

Aspergillus megasporus was isolated from Canadian house dust collected in Wolfville, Nova Scotia and Little Lepreau, New Brunswick, and was also isolated from chocolate butter in the Netherlands. Phylogenetically, the position of this species varies depending on which gene is analysed; *CaM* resolves it in its own distinct clade, *BenA* in a clade with a poorly supported branch with *A. glaucus* and *A. proliferans*, and *RPB2* closest to *A. niveoglaucus*. The multigene phylogeny places it in a large clade, including *A. brunneus*, *A. niveoglaucus*, *A. neocarnoyi*, *A. glaucus* and *A. proliferans*. Both *A. niveoglaucus* and *A. megasporus* produces conidia respectively reaching 11 and 12 µm, easily distinguishing them from other species of section *Aspergillus*. *Aspergillus megasporus* can be distinguished from *A. niveoglaucus* based on its faster growth on DG18. *Aspergillus megasporus* produces extrolites commonly detected in species of section *Aspergillus*, including echinulin, neoechinulin and preechinulin. However, we also detected quinolactacin, a first report for the group. In an independent study using different methods and media, compounds detected from CBS 141772 include asperflavin, auroglaucin, bisanthrons, dihydroauroglaucin, echinulin, emodin, erythroglaucin, flavoglaucin, isoechinulins, neoechinulins, preechinulin, physcion, quinolactacin, tetracyclic compounds, and tetrahydroauroglaucin (Frisvad, personal communication).

Aspergillus mallochii was isolated from pack rat dung collected from San Mateo, California, USA. An additional strain was recently isolated from '*Chocolat miroir*' icing for a cake in the Netherlands. Phylogenetically, it has *A. appendiculatus* as sister species, originally described by Blaser (1975) from German smoked sausages. These two species share identical ITS sequences that are distinct from all others in the section (Fig. 2). All other genes, especially *RPB2*, easily distinguish the two. Morphologically

they are also similar, but the roughened ascospores of *A. mallochii* are distinct from the smoother ascospores of *A. appendiculatus*. In an independent study using different methods and media, compounds detected from CBS 141776 included auroglaucin, dihydroauroglaucin, echinulins, erythroglaucin, flavoglaucin, isoechinulins, neoechinulins, tetracyclic compounds and tetrahydroauroglaucin. Comparisons revealed that *A. appendiculatus* produced several compounds not observed in *A. mallochii*, such as asperflavin, asperentins, bisanthrons, 5-farnesyl-5,7-dihydroxy-4-methylphthalide, mycophenolic acid, physcion and questin (Nielsen et al. 2011). None of the extrolites identified in *A. mallochii* are unique to the species.

Quinolactacin A1, A2 & B were the major compounds produced by *A. megasporus*, the only species of section *Aspergillus* that produces these. These quinolone structures with a γ-lactam ring were first characterized from fermentations of an unknown *Penicillium* species (Kakinuma et al. 2000; Takahashi et al. 2000) and further characterized by Kim et al. (2001) in *Penicillium citrinum*, where they were demonstrated to be acetylcholinesterase inhibitors. Quinolactacins have since been reported from multiple *Penicillium* species from sections *Citrina* (Houbraken et al. 2011), *Brevicompacta* (Frisvad et al. 2013; Perrone et al. 2015) and *Robsamsonia* (Houbraken et al. 2016); *Aspergillus quadricinctus*, *A. stramenius* (section *Fumigati*) (Frisvad and Larsen 2015b; Samson et al. 2007), *A. karnatakaensis* (section *Aenei*) (Varga et al. 2010); and from the distantly related marine derived *Xylariaceae* (Nong et al. 2014). Based on current knowledge, the echinulins (including echinulin, neoechinulin and isoechinulin) detected in both *A. megasporus* and *A. mallochii* seem specific to *Aspergillus* sections *Aspergillus* and *Restricti* (Frisvad and Larsen 2015a). Echinulin was first discovered in *A. brunneus* (= *E. echinulatum*) by Quilico and Panizzi (1943). It was subsequently detected in many more section *Aspergillus* species (Ali et al. 1989; Almeida et al. 2010; Greco et al. 2015; Li et al. 2008b; Slack et al. 2009; Smetanina et al. 2007; Vesonder et al. 1988) and shown to be toxic to animal cells (Ali et al. 1989; Umeda et al. 1974), while swine and mice respectively refused feed and water containing echinulin (Vesonder et al. 1988). The presence of echinulin in the environment is not well documented however. In contrast to the negative effects of echinulin, neoechinulin has anti-oxidant properties (Yagi and Doi 1999) and protected PC12 cell lines, used in neurological research, against cell death by peroxynitrite (Kimoto et al. 2007; Maruyama et al. 2004).

Visagie et al. (2014a) emphasized that despite the existence of comprehensive ITS barcode reference databases, this marker is insufficient for identifying most *Aspergillus*, *Penicillium* and *Talaromyces* to species level in culture-independent surveys such as those of Amend et al. (2010) and Adams et al. (2013a; 2013b). The reference sets include sequences for multiple genes obtained from ex-type cultures for all accepted species in these genera (Samson et al. 2014; Visagie et al. 2014b; Yilmaz et al. 2014) and are invaluable as anchoring points for species. Curating databases is laborious and has many complications, but both UNITE and NCBI have ongoing curation projects involving ITS barcodes (Kõljalg et al. 2013; Nilsson et al. 2015; Schoch et al. 2014). ITS will always have limited resolution for species identification. In *Aspergillus* and *Penicillium*, ITS is highly conserved in many sections, as is observed in our phylogeny

of section *Aspergillus* (Fig. 2). Barcode-based metagenomic studies commonly use Last Common Ancestor (LCA) analyses for assigning OTU's to GenBank taxonomic nodes. In LCA, when the analysis cannot identify an operational taxonomic unit (OTU) at a taxonomic rank, it will move up one level until it can make a confident assignment. As now implemented, most species of sect. *Aspergillus* species would be identified only to the generic level. For species-rich genera such as *Aspergillus* and *Penicillium*, this is problematic. Different ecologies, functions, extrolites etc. are often associated with specific groups (i.e. true xerophily in at least three sections of *Aspergillus*), and much potentially important information is lost because of this imprecision. To circumvent this problem, a few recent studies have used alternative genes combined with next generation sequencing (NGS) for making "mass" identifications in *Aspergillus*. Lee and Yamamoto (2015) assessed the accuracy of high-throughput amplicon sequencing using ITS, *BenA* and *CaM*, and identified OTU's using the ex-type sequences published by Samson et al. (2014). Results were promising with both *BenA* and *CaM*, which are obviously more accurate than ITS. Unfortunately, amplifications of these alternative barcodes were sometimes problematic, perhaps because they are single copy genes or undocumented sequence variation, especially considering comparisons to only ex-type sequences. Similar results were obtained in a subsequent study by Lee et al. (2016). Even though these types of studies are promising, considerable optimisation is required to amplify and sequence low copy markers from a complex matrix, and shotgun sequencing may be more effective. No matter what the experimental approach used by ecologists, taxonomists need to make identifications as easy as possible, not only in the traditional morphological sense, but also by generating reference data that will enhance the robustness of analyses of data generated using new technologies such as NGS. Surveys such as ours are thus important not only for discovering previously unnamed species, but for providing more reference sequences in public databases that capture infraspecies sequence variation for multiple barcodes.

Recently, the International Code of Nomenclature for algae fungi and plants (ICN, Melbourne Code; (McNeill et al. 2012)) adopted single name nomenclature for pleomorphic fungi, meaning decisions are needed to choose either the teleomorphic (sexual morph) or anamorphic (asexual morph) name to represent the genus. In anticipation of this change, Houbraken and Samson (2011) reviewed the taxonomy and phylogeny *Trichocomaceae*, of which *Penicillium* and *Aspergillus* are the largest groups, using a four gene combined analysis. The situation with the generic concept and name for *Aspergillus* is complicated and controversial, partly because of conflicting interpretations of phylogenies, and partly because of differing opinions on how much taxonomic weight to apply to sexual states in generic concepts (Houbraken and Samson 2011; Pitt and Taylor 2014; Taylor et al. 2016). In this paper, we have followed the traditional broad concept of *Aspergillus* advocated by the International Commission of *Penicillium* and *Aspergillus* (ICPA), which includes species formerly classified in the sexual genera *Eurotium*, *Emericella*, *Neosartorya* and *Petromyces* in *Aspergillus*. The section of *Aspergillus* that is the focus of our paper includes the type species of both *Aspergillus* (*A. glaucus*) and *Eurotium* (*E. herbariorum*). With the community decision

to respect the priority of *Aspergillus* in both the nomenclatural and practical sense, the new species described here would be described in *Aspergillus* whether a broad or narrow generic concept is applied. The recent proposal by Taylor et al. (2016) to conserve *Aspergillus* with the type species changed to *A. niger* is still being discussed, but at this time seems unlikely to be accepted. If the proposal is implemented, along with the narrower generic concept endorsed by these authors, approximately 180 *Aspergillus* species would be renamed, including those described in this paper.

Acknowledgements

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Supplementary material 1

Species isolated from house dust using selective xerophilic media

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Data type: Occurrence, GenBank info

Explanation note: Species isolated from house dust using selective xerophilic media, their occurrence and GenBank numbers for sequences generated for these strains.

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